Variation in Aggressiveness Among Isolates of *Cercospora* from Maize as a Potential Cause of Genotype–Environment Interaction in Gray Leaf Spot Trials

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ABSTRACT

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The use of genetically resistant maize hybrids is the preferred means of control of gray leaf spot, caused by *Cercospora zeae-maydis*. One problem faced by maize breeders attempting to breed for resistance to gray leaf spot is the high degree of genotype–environment interactions observed in disease trials. In North Carolina gray leaf spot trials conducted at four locations in the western part of the state, we found consistent hybrid–location interactions over the 1995 and 1996 growing seasons. Isolates of *C. zeae-maydis* from those test locations were evaluated on the same hybrids used in the multilocation testing at a location in central North Carolina that does not have a history of gray leaf spot. The hybrid–isolate interactions observed in the isolate trial mirrored the hybrid–location effects seen in the multilocation testing. Most of the interactions arose from changes in the magnitude of differences between hybrids when inoculated with the isolates rather than from any change in hybrid ranking. Analysis of internal transcribed spacer-restriction fragment length polymorphisms (RFLPs) and mitochondrial rDNA RFLPs of those isolates and others revealed that both type I and type II sibling species of *C. zeae-maydis*, as well as *C. sorghi* var. *maydis,* are isolated from typical gray leaf spot lesions. Breeders should use the most aggressive isolates of *C. zeae-maydis* to maximize discrimination between genotypes in gray leaf spot trials.

Gray leaf spot (GLS) is considered to be the most important foliar disease of maize in the United States. The disease was originally described from southern Illinois in 1925 by Tehon and Daniels (25) but, for many years, was considered to be a relatively obscure and unimportant disease, chiefly confined to the southern Appalachian region (20,21). During the 1970s, GLS increased in incidence and severity in this region in conjunction with the increased adoption of conservation tillage practices to reduce soil erosion (9,16-19,22,24). The disease has since spread and increased in incidence and severity as reduced tillage practices became commonplace throughout the eastern and midwestern maize-growing regions. GLS can now routinely be found in most of the United States maize production areas from eastern Colorado (under center pivot irrigation) to coastal regions of North Carolina and Virginia (2,11,16).

Gray leaf spot is caused by the imperfect fungus, *Cercospora zeae-maydis* Tehon & E. Y. Daniels, although *C. sorghi* var. *maydis* also is listed as being associated with GLS lesions (5). Recently, it has been shown that *C. zeae-maydis* is composed of two genetically distinct but morphologically similar sibling species (4,11,12). The more common sibling species (type I) is widely distributed in the United States, whereas the second sibling species (type II) is confined to the eastern United States, although both species may be found in the same field. Variability in pathogenic aggressiveness, growth rate, colony morphology, the presence and relative abundance of spermogonia, and size of spermatia has been reported among isolates of *C. zeae-maydis* (11,15,16,28). Although they found a wide range in aggressiveness among isolates, Bair and Ayers (1) did not find any evidence of specificity or races of *C. zeae-maydis*, as evidenced by a lack of a significant hybrid–isolate interaction.

Genetic resistance is the primary means of control of GLS in the United States. Several sources of resistance have been found among temperate and tropical germ plasm, and this resistance has been incorporated into several maize breeding programs, including the maize breeding program at North Carolina State University (3,4,6–8,10,14,23,26,27). Commercial hybrids with suitable maturity and adaptation to North Carolina having acceptable levels of GLS resistance are available. Most GLS resistance in commercial hybrids is coming from the male inbred line parent (or 'Lancaster') side of the hybrid pedigree. A recurring problem in GLS trials in North Carolina has been the variable results often obtained from different test locations in the western mountains of the state. This high degree of genotype–environment interaction has complicated breeding for resistance as well as the quantitative trait loci mapping studies of GLS resistance (3,6,23). In particular, we have noticed that certain hybrids appear to have resistance equal to that of the most resistant commercial hybrid when tested at locations in the northern mountains, but are badly blighted when tested at locations farther south. The objectives of this study were to (i) determine how much, if any, of the observed hybrid–environment interaction in GLS ratings is due to differences in aggressiveness among isolates of the pathogen at those locations and (ii) determine the extent of molecular variation among those isolates.

MATERIALS AND METHODS

Field trials. A set of 10 hybrids (DK683, DK689, Pioneer hybrid 3394, Agratech 967, SS883a, SS943, Deltapine 4820, Cargill 8936, B73xMo17, and FFR406.411xLH214) representing a range in GLS reactions was evaluated in field trials at four locations in the mountains of North Carolina (Laurel Springs, Hendersonville, Andrews, and Marion). Trials consisted of randomized complete blocks with two replications planted during May 1995 and May 1996. Experimental units consisted of single rows, 4 m long and spaced 0.95 m apart, with 25 seeds planted per plot. Plots were not thinned. Plots at Laurel Springs were inoculated with an oat grain culture of an isolate of *C. zeae-maydis* originally from Ohio and obtained from Syngenta Seeds (Golden Valley, MN; courtesy R. Kuznia). At other locations, we relied upon naturally occurring inoculum to initiate GLS epidemics. Whole plots were visually rated for total leaf area damaged at multiple times late in the grain-filling period (August to September) each year using a 1-to-9 scale where 9 = no disease, 8 = 1 to 10%, 7 = 10 to 20%, 6 = 20 to 40%, 5 = 40 to 60%, 4 = 60 to 80%,

3 = 80 to 90%, 2 = 90 to 99%, and 1 = 100% GLS severity (no green leaf tissue remaining). The actual number of rating times varied at each location and each year due to the logistics of travel to these distant locations. Data were analyzed in a combined (over location and years) analysis of variance (ANOVA) using early and late season ratings. Disease ratings were selected as "early" or "late" based on GLS development and crop maturity in each environment. Early ratings were the first ratings at which distinct differences in GLS reaction were noted between known susceptible and resistant genotypes. Late ratings were the latest ratings at which those genotypic differences were still apparent, but before hybrids had begun senescing. Hybrids and locations were considered fixed variables and years were considered random. Main effects and interactions were tested for significance using the appropriate F tests in the ANOVA. Hybrid means at each location in each year were compared using Fisher's least significant difference at the $P = 0.05$ level of probability.

The same hybrids were evaluated against six isolates of *C. zeae-maydis* obtained from symptomatic leaf tissue collected from the North Carolina test sites (Laurel Springs, Andrews, Hendersonville, and Marion) and elsewhere (Salisbury, NC and Edgar County, IL) in the fall of 1995. The trial was conducted at the Central Crops Research Station on the upper coastal plain of North Carolina at Clayton over the 1996 and 1997 growing seasons. This research station does not have a history of GLS and the fields used were "clean tilled" and had not been cropped in maize for the two previous growing seasons. The field trial consisted of a split plot design with three replications arranged in a randomized complete block design, with isolates constituting whole plots and the 10 hybrids constituting subplots. Whole plots were separated from each other by at least 2 m of uninoculated border plots of the highly resistant hybrid DK683. Plots were inoculated twice with a sorghum grain culture of the six isolates, at the four-to-six- and eight-to-ten-leaf stages of devel-

opment. The sorghum grain inoculum was prepared by soaking sorghum seed overnight in tap water, draining off excess water, dispensing the soaked grain into 1-liter flasks, and autoclaving for 1 h. Flasks were inoculated with 20 ml of a slurry made by agitating 10- to 14-day-old V8 agar cultures of the isolates in sterile water with 5-mm glass beads. Cultures were maintained in 50% glycerol at –80°C. Plots were rated for percent GLS severity five times, beginning at midanthesis. Area under the disease progress curves were calculated using the method of Wilcoxon et al. (30). Data from the last rating period were omitted from the analyses because of extensive interplot spread of isolates.

Molecular marker studies. Genetic analysis of internal transcribed spacer (ITS) regions of nuclear rDNA using restriction fragment length polymorphism (RFLP) analysis of the ITS1/ITS4 polymerase chain reaction (PCR) product and portions of mitochondrial (mt)-rDNA (both large and small subunits, using ML7/ML8 and MS1/MS2 primer sets, respectively) (29) were carried out on the six isolates of *C. zeae-maydis* used in the field study along with six other isolates of *Cercospora* isolated from maize in North Carolina, Minnesota, Illinois, Iowa, and Kenya. Total DNA was extracted from potato dextrose agar cultures of the isolates using a modification of the protocol of Duteau and Leslie (13). Modifications included a different lysis buffer (100 mM LiCl; 10 mM EDTA, pH = 8.0; 10 mM Tris, pH = 7.4; and 0.5% sodium dodecyl sulfate), the use of phenol:chloroform:isoamyl alcohol (vol/vol/vol) instead of phenol for extractions, and the addition of RNAse at a final concentration of 1 μg/μl at the end of the procedure. The ITS1/ITS4, ML7/ML8, and MS1/MS2 regions were amplified via PCR reactions in 50-μl total reaction volume with 5 μl of Promega 10× buffer, 8 μl of dNTPs (1.25 mM each), 2.5 μl each of the primers (10-μM stock), 1.25 units of Taq polymerase (Promega Corp., Madison, WI), 10 μl of template DNA (0.1 ng/μl), either 3 μl (ITS1/ITS4) or 6 μl (ML7/ML8 and MS1/MS2) of 25 mM $MgCl_2$, and sterile dH_2O to volume. PCR was conducted in a

model PTC-100 Thermocycler (MJ Research, Watertown, MA) set to the following program: 94°C for 3 min., followed by 35 cycles of 94°C for 1 min, 50°C for 30 s, ramping to 72°C (1°C/s) and holding for 1 min. After 35 cycles, there was a final extension time of 7 min at 72°C followed by a holding temperature of 4°C. Reaction products (4 μl of reaction product plus 2 μl of loading dye) were loaded into wells of 1% agarose gels in 1× Tris-borate EDTA (TBE) containing ethidium bromide. Gels were electrophoresed at 75 V for 4 to 5 h. A lambda *Eco*RI-*Hind*III ladder was included in one lane to estimate size of reaction products. Reaction products also were digested with nine different restriction enzymes (*Alu*I, *Ava*II, *Dde*I, *Hae*II, *Hinf*I, *Hha*I, *Sau3*AI, *Taq*I, and *Tru*9I) following the directions given by enzyme manufacturers. Restriction digests (20 μl) along with 5 μl of loading dye were loaded into wells in a 3% agarose gel in 1× TBE containing ethidium bromide and electrophoresed at 75 V for 4 to 5 h. A ΦX174/*Hae*III ladder was loaded into one lane and used to estimate sizes of restriction fragments.

RESULTS

Field trials. The reactions of the 10 maize hybrids to GLS varied depending on location, year, and rating date. When the data were combined over years, the hybrid–location interaction term was highly significant ($P < 0.001$) at both rating periods, indicating that the resistance of the hybrids relative to each other varied significantly depending upon the location of the trial (Table 1). The year–location effect also was highly significant ($P < 0.001$), but the hybrid–location–year effect was not significant, indicating that the relevant hybrid–location effect was consistent over both years of the trial. The year–hybrid interaction term was significant ($P < 0.05$) for the late GLS ratings only, but this effect was small in relation to the hybrid–location effect. Some of the hybrid–location interaction appears to have been due to changes in the magnitude of the differences among hybrids between locations. For example, Cargill 8936 was significantly less resistant than the highly resistant hybrids Dekalb DK683 and DK689 at both rating dates at Andrews, Hendersonville, and Marion, but not at Laurel Springs (Table 2). Similarly, FFR406.411xLH214 was significantly more susceptible than DK683 at late rating periods at Andrews, Hendersonville, and Marion, but not so at Laurel Springs. There were also a few examples of significant reversals in hybrid rankings between locations. When data from early rating periods are compared, four hybrids (Agratech 967, FFR406.411xLH214, and Southern States SS883A and SS943) were significantly less resistant than DK683 at Andrews, but were significantly more resistant at Laurel Springs.

Table 1. Combined analyses of variance for early and late season gray leaf spot reactions for 10 maize hybrids evaluated at four North Carolina locations over two years[z]

Source	df	Mean square	
		Early	**Late**
Year	1	0.23	4.90
Hybrid	9	15.80***	34.78***
Location	3	49.84	73.82
Rep(year–location)	11	2.28	13.52
Year–location	3	7.14***	48.21***
Year–hybrid	9	0.34	1.91*
Hybrid–location	27	1.34***	2.24***
Hybrid–year–location	27	0.31	0.51
Pooled error	72	0.24	0.37

[z] Rated on a 1-to-9 scale where 1 = 100% leaf area blighted and 9 = no disease); * and *** indicate mean square is significant at the $P = 0.05$ or 0.001 level of probability, respectively.

Despite these examples, there was some consistency in the GLS ratings among locations. The two susceptible hybrids, B73xMo17 and Pioneer hybrid 3394, were consistently judged equivalent and the most susceptible at all locations at all rating dates. The two Dekalb hybrids were also among the most resistant hybrids at all locations and rating periods, with the exception of early ratings at Laurel Springs. The responses of the remaining intermediate hybrids were the most variable, particularly when compared with either the consistently resistant or susceptible hybrids mentioned above.

When isolates of *C. zeae-maydis* from the North Carolina test locations, along with isolates from Salisbury, NC and Edgar County, IL, were tested on the 10 hybrids at Clayton, NC, significant ($P < 0.05$) hybrid–isolate interactions were detected when the data were combined over years. However, this effect was small in comparison to main effects and the year–isolate and year–hybrid interactions ($P < 0.001$). Isolates varied significantly in their aggressiveness, with the Laurel Springs and Hendersonville isolates being the least and an isolate from Marion the most aggressive (Table 3). The hybrid–isolate interactions appear to arise from changes in the magnitudes of the differences between hybrids

with different isolates rather than from any significant changes in hybrid rankings. Some of the interactions were similar to those observed from our location trials. For example, Cargill 8936 was significantly less resistant than DK683 when tested with isolates from Andrews and Marion, but appeared equal in resistance when evaluated with less aggressive isolates from Hendersonville and Andrews. There was less discrimination among the hybrids when they were inoculated with these less-aggressive isolates. When inoculated with the isolate from Laurel Springs, only two groupings of hybrids resulted; the two susceptible hybrids, B73xMo17 and Pioneer hybrid 3394, were significantly different from the other eight hybrids. Similarly, the isolate from Hendersonville was only able to resolve the hybrids into three groups; B73xMo17 was more susceptible than Pioneer hybrid 3394, and the remaining eight hybrids formed the third group. In contrast to the results from the location trials where B73xMo17 and Pioneer hybrid 3394 were judged equally susceptible, all the isolates except the Laurel Springs isolate were able to discriminate between these two hybrids.

Molecular analysis. All isolates produced an amplified fragment of identical length when the ITS1/ITS4 primers were

used. When this fragment was digested with restriction enzymes, polymorphisms were observed with six of the nine restriction enzymes tested. The ITS1/ITS4 fragment from the Laurel Springs isolate was differentiated from the other isolates by digestion with *Dde*I, *Hae*II, and *Hha*I. Digestion with *Alu*I distinguished the ITS1/ITS4 fragment of the Davidson County, NC and Kenya isolates from the remaining isolates, whereas digestion with *Tru*9I separated the Laurel Springs, Davidson County, and Kenya isolates from the others. RFLPs from digestion of the ITS1/ITS4 fragment with *Taq*I separated the isolates into three groups: the Laurel Springs isolate was the sole representative of one group, the Davidson County and Kenya isolates composed the second group, and the remaining isolates constituted the third group (Fig. 1). Based on the *Taq*I restriction patterns, the three isolate groups represent *C. zeae-maydis* type II, *C. sorghi* var. *maydis*, and *C. zeae-maydis* type I, respectively, as shown in Wang et al. (28). These three species groups also were distinguished when the size of the ML7/ML8 fragment amplified from the large subunit of mt-rDNA was measured (Fig. 2). There was further discrimination among the two *C. sorghi* var. *maydis* isolates because the Davidson County isolate

Table 2. Mean gray leaf spot ratings for 10 hybrids evaluated at four locations in western North Carolina in 1995 and 1996[z]

	Location and rating period							
	Andrews		Hendersonville		Laurel Springs		Marion	
Hybrid	Early	Late	Early	Late	Early	Late	Early	Late
Agratech 967	5.75 cd	5.50 ab	8.50 a	6.25 a	9.00 a	7.75 a	5.75 ab	2.25 de
B73xMo17	4.00 e	2.50 c	6.00 c	2.25 b	5.25 e	2.25 c	4.75 d	1.00 e
Cargill 8936	5.25 cd	4.00 bc	7.50 b	3.25 b	7.50 cd	5.75 b	5.00 cd	2.25 de
Dekalb DK683	7.50 a	6.75 a	8.50 a	6.50 a	7.25 d	6.50 ab	6.25 a	5.50 a
Dekalb DK689	6.75 ab	5.75 a	8.00 ab	5.25 a	7.50 cd	6.75 ab	6.25 a	4.25 ab
Deltapine 4820	6.75 ab	6.75 a	8.00 ab	5.50 a	8.00 bc	6.50 ab	5.50 bc	4.00 abc
FFR406.411XLH214	5.00 d	4.00 bc	8.25 ab	5.25 a	8.50 ab	7.50 a	5.75 ab	2.50 cde
Pioneer 3394	4.00 e	2.50 c	6.00 c	2.50 b	5.00 e	3.50 c	3.75 e	1.00 e
Southern States SS883A	5.50 cd	5.25 ab	8.00 ab	5.50 a	8.50 ab	7.50 a	6.25 a	2.00 de
Southern States SS943	6.00 bc	5.50 ab	8.25 ab	6.75 a	8.75 a	8.00 a	6.00 ab	3.25 bcd

[z] Rated on a 1-to-9 scale where 1 = 100% leaf area blighted and 9 = no disease. Means followed by the same letter within a column are not significantly different at the $P = 0.05$ level of probability based on Fisher's least significant difference statistics. Disease ratings were selected as "early" or "late" based on gray leaf spot development and crop maturity in each environment.

Table 3. Mean area under the disease progress curves of 10 maize hybrids inoculated with six isolates of *Cercospora* over 2 years at Clayton, NC[y]

Hybrid	Andrews, NC	Edgar, IL	Hendersonville, NC	Laurel Springs, NC	Marion, NC	Salisbury, NC	Hybrid means
Agratech 967	341 e	272 de	229 c	177 b	589 e	483 de	349 e
B73xMo17	1,294 a	1,203 a	921 a	755 a	1,596 a	1,518 a	1,215 a
Cargill 8936	662 c	604 c	299 c	299 b	864 c	703 c	572 c
Dekalb DK683	258 e	211 e	236 c	177 b	593 e	431 e	318 e
Dekalb DK689	260 e	285 de	199 c	163 b	693 cde	400 e	333 e
Deltapine 4820	430 de	440 cd	318 c	267 b	605 e	558 cde	436 cde
FFR406.411XLH214	607 cd	413 cd	335 c	218 b	1,042 c	633 cd	541 cd
Pioneer 3394	1,022 b	1,002 b	720 b	563 a	1,300 b	1,203 b	968 b
Southern States SS883A	417 de	299 de	284 c	223 b	827 cd	546 cde	433 cde
Southern States SS943	421 de	385 de	301 c	169 b	657 de	442 de	396 de
Isolate means[z]	571 abc	511 abc	384 bc	301 c	877 a	692 ab	…

[y] Hybrid means are the mean of eight observations. Means within the same column followed by the same letter are not significantly different at the $P = 0.05$ level of probability based on Fisher's least significant difference (LSD) statistics.

[z] Isolate means are the mean of 80 observations. Means followed by the same letter are not significantly different at the $P = 0.05$ level of probability based on Fisher's LSD statistics.

yielded two distinct bands when the ML7/ML8 primers were used, the largest of which it shared in common with the Kenya isolate. Digestion of the ML7/ML8 fragments with restriction enzymes revealed no further degree of polymorphism among the isolates. Amplification of the small subunit of mt-rDNA using the MS1/MS2 primers resulted in three distinct fragments for all the isolates (Fig. 3). When these fragments were restriction digested with *Dde* and *Hha*I, the two isolates of *C. sorghi* var. *maydis* could be distinguished from the remaining isolates, but no polymorphisms between the sibling species of *C. zeae-maydis* were observed with any of the nine restriction enzymes tested.

DISCUSSION

Genotype–environment interactions complicate breeding and selection for any trait, including disease resistance. The usual approach to increase the efficiency of selection in such situations is to increase the number of environments in which the material is evaluated, greatly increasing the cost and time required. An alternative approach is to find or create an environment in which the trait of interest is consistently expressed and allows maximum discrimi-

Fig. 1. Restriction fragments generated when the internal transcribed spacer (ITS)l/ITS4 amplified fragment from 11 isolates of *Cercospora* associated with gray leaf spot of maize was digested with four restriction enzymes. Lanes were loaded in identical order both top and bottom. Lane 1 = •X174/*Hae*III ladder; lanes 2 and 14 = Andrews, NC isolate; 3 and 15 = Davidson County, NC; lanes 4 and 16 = Hendersonville, NC; lanes 5 and 17 = Edgar County, IL; lanes 6 and 18 = IL-NK; lanes 7 and 19 = Kenya; lanes 8, 9, 20, and 21 = Laurel Springs, NC; lanes 10 and 22 = Marion, NC; lanes 11 and 23 = Minnesota; 12 and 24 = Salisbury, NC; and lanes 13 and 25 = IA-001. Lanes 2–13 (top) were digested with *Dde*I; lanes 14–25 (top) with *Alu*I; lanes 2–13 (bottom) with *Hae*II; and lanes 14–25 (bottom) with *Taq*I. Fragments were electrophoresed at 75 V for 4 to 5 h on 3% agarose in I× Tris-borate EDTA containing ethidium bromide.

nation among genotypes. In our situation of screening maize germ plasm for GLS resistance in North Carolina, most of the genotype–environment interaction appears to result from changes in the magnitude of differences among hybrids between location, rather than changes in rankings. Greater discrimination between levels of resistance among hybrids was observed at the Andrews and Marion locations, where disease levels are generally higher than at Hendersonville and Laurel Springs. This was particularly evident among hybrids considered intermediate in resistance to GLS, such as Cargill 8936. When isolates from the four test locations were compared in a common environment, significant differences in aggressiveness of those isolates were apparent. The least aggressive isolates were least able to discriminate among levels of resistance in our set of hybrids. The significant hybrid–isolate interaction resulted from changes in the magnitude of the differences among hybrids when inoculated with the isolates and not from changes in hybrid ranking. There is no evidence for pathogen races. Although the hybrid–isolate interactions tended to reflect the interaction of hybrids with locations from which the isolates were collected, it would be an oversimplification to state that hybrid–location interactions arise solely from differences among populations of the pathogen at those different locations. We only tested single isolates of the pathogen from each location. With the possible exception of the situation at Laurel Springs, it is unlikely that the local populations of *C. zeae-maydis* at the remaining three locations are represented by the isolates used in our test at Clayton. The isolate obtained from inoculum used at Laurel Springs may in fact represent that population. It was the only

Fig. 2. Large subunit mitochondrial rDNA fragments polymerase chain reaction amplified using the ML7/ML8 primers from genomic DNA of 11 isolates of *Cercospora* associated with gray leaf spot of maize. Lane 1 = •X4/*Hae*III ladder; lane 2 = Andrews, NC isolate; lane 3 = Davidson County, NC; lane 4 = Hendersonville, NC; lane 5 = Edgar County, IL; lane 6 = IL-NK; lane 7 = Kenya; lanes 8 and 9 = Laurel Springs, NC; lane 10 = Marion, NC; lane 11 = Minnesota; lane 12 = Salisbury, NC; and lane 13 = IA-00l. Fragments were electrophoresed at 75 V for 4 to 5 h on 3% agarose in 1× Tris-borate EDTA containing ethidium bromide.

location where artificial inoculum was used and the likelihood of the successful overwintering of a native population of the pathogen was severely reduced due to the removal of crop residues by silage harvesting and moldboard plowing of those remaining.

At least three distinct species of *Cercospora* are routinely isolated from GLS lesions on maize in North Carolina: *C. zeae-maydis* types I and II (28) and *C. sorghi* var. *maydis* The three species are genetically distinct as shown by ITS RFLPs and by the size of ML7/ML8 amplified fragments of large subunits of mt-rDNA. It is tempting to relate hybrid–isolate interactions to differences between the two sibling species of *C. zeae-maydis*. The Laurel Springs isolate was the sole representative of *C. zeae-maydis* type II in our trial and was also the least aggressive isolate. However, a range of aggressiveness has been found in both sibling species of *C. zeae-maydis* (11, *unpublished data*); therefore, the low aggressiveness of the Laurel Springs isolate is not typical of *C. zeae-maydis* type II. We also have recovered isolates of *C. sorghi* var. *maydis* from GLS samples from several locations in North Carolina and Kenya. The role this species may play in GLS of maize is not clear. It appears to be closely related to *C. sorghi* (5,28) but there may be significant variation among isolates of *C. sorghi* var. *maydis,* as indicated by a difference in the number of fragments amplified with the ML7/ML8 primer pair in the Davidson County, NC isolate versus the Kenya isolate.

Although genotype–location interactions and, to a lesser extent, genotype–isolate interactions will remain a feature of GLS trials, these data support the idea that some
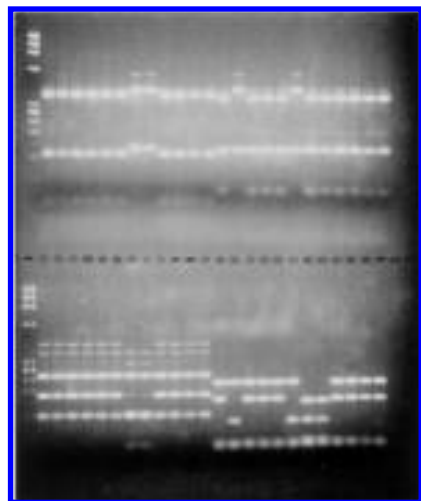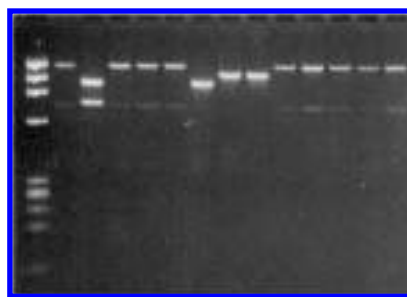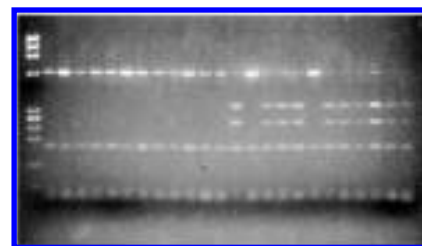

Fig. 3. Small subunit mitochondrial rDNA fragments polymerase chain reaction amplified using the MS1/MS2 primers from genomic DNA of 11 isolates of *Cercospora* associated with gray leaf spot of maize (lanes 2–13). Those same fragments following digestion with *Hha*I are shown in lanes 14–25. Lane 1 = •X4/*Hae*III ladder; lanes 2 and 14 = Andrews, NC isolate; 3 and 15 = Davidson County, NC; lanes 4 and 16 = Hendersonville, NC; lanes 5 and 17 = Edgar County, IL; lanes 6 and 18 = IL-NK; lanes 7 and 19 = Kenya; lanes 8, 9, 20, and 21 = Laurel Springs, NC; lanes 10 and 22 = Marion, NC; lanes 11 and 23 = Minnesota; 12 and 24 = Salisbury, NC; and lanes 13 and 25 = IA-001. Fragments were electrophoresed at 75 V for 4 to 5 h on 3% agarose in 1× Tris-borate EDTA containing ethidium bromide.

of this interaction is the result of less than optimal disease development at some locations which may result from a less favorable environment for disease development or reduced aggressiveness of the pathogen population at that location. There is no evidence for the existence of physiological races within sibling species of *C. zeae-maydis*, nor is there evidence of specificity of resistance in maize to the two sibling species. One approach to reduce genotype–environment interaction and to maximize gain from selection for GLS resistance is to use the mostly highly aggressive isolates of the pathogen in a favorable location when establishing disease trials. We have found that aggressiveness of *C. zeae-maydis* (both types I and II) is a reproducible trait, is easily selected, and is expressed in locations less than ideal for GLS development, such as those at our Clayton location on the upper coastal plain of North Carolina.

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